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Mucosal Vaccination with Formalin-Inactivated Avian Metapneumovirus Subtype C Does Not Protect Turkeys Following Intranasal Challenge

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SUMMARY. Studies were performed to determine if mucosal vaccination with inactivated avian metapneumovirus (aMPV) subtype C protected turkey poults from clinical disease and virus replication following mucosal challenge. Decreases in clinical disease were not observed in vaccinated groups, and the vaccine failed to inhibit virus replication in the tracheas of 96% of vaccinated birds. Histopathologically, enhancement of pulmonary lesions following virus challenge was associated with birds receiving the inactivated aMPV vaccine compared to unvaccinated birds. As determined by an enzyme-linked immunosorbent assay (ELISA), all virus-challenged groups increased serum immunoglobulin (Ig) G and IgA antibody production against the virus following challenge; however, the unvaccinated aMPV-challenged group displayed the highest increases in virus-neutralizing antibody. On the basis of these results it is concluded that intranasal vaccination with inactivated aMPV does not induce protective immunity, reduce virus shedding, or result in decreased histopathologic lesions.

RESUMEN. La vacunación mucosal con metaneumovirus aviar subtipo C inactivado con formalina no protege a los pavos de un desafío intranasal.

Se realizaron estudios para determinar si la vacunación mucosal con metaneumovirus aviar subtipo C inactivado con formalina protege pavitos de la enfermedad clínica y replicación viral luego de un desafío mucosal. No se observaron disminuciones en la clínica de la enfermedad en los grupos vacunados y la vacuna no inhibió la replicación viral en la tráquea del 96% de las aves vacunadas. Histopatológicamente, se asoció una aumento de las lesiones pulmonares posteriores al desafío con aves que recibieron la vacuna de metaneumovirus aviar inactivada, en comparación con las aves no vacunadas. Según se determinó mediante una prueba de inmunoensayo asociado a enzimas, todos los grupos desafiados incrementaron la producción de inmunoglobulina G y de inmunoglobulina A contra el virus de desafío. Sin embargo, el grupo no vacunado desafiado con metaneumovirus aviar mostró el mayor incremento en anticuerpos neutralizantes. Sobre la base de estos resultados se concluye que la vacunación intranasal con metaneumovirus aviar subtipo C inactivado no induce inmunidad protectiva, no reduce la diseminación del virus ni resulta en una disminución de las lesiones histopatológicas.

Key words: avian metapneumovirus, inactivated vaccine, turkeys, mucosal vaccination

Abbreviations: aMPV = avian metapneumovirus; BCA = bicinchoninic acid; bRSV = bovine respiratory syncytial virus; CPE = cytopathic effect; DMEM = Dulbecco's modified eagle's medium; ELISA = enzyme-linked immunosorbent assay; hMPV = human metapneumovirus; hRSV = human respiratory syncytial virus; Ig = immunoglobulin; IN = intranasal; Mn = Minnesota; OD = optical density; PBS = phosphate-buffered saline; PBS-T = PBS containing Tween 20; pc = postchallenge; RT-PCR = reverse-transcription polymerase chain reaction; SPF = specific pathogen free; $TCID_{50}$ = mean tissue-culture infectious dose; TRT = turkey rhinotracheitis; VI = virus isolation; VN = virus neutralization

Avian metapneumovirus (aMPV) is the causative agent of turkey rhinotracheitis (TRT), which results in primarily a respiratory disease (5,9). aMPV belongs to the *Pneumovirinae* subfamily of the *Paramyxoviridae* family and contains a negative-sense, nonsegmented, single-strand RNA genome of approximately 13.3 kilobases that code for eight genes.

Clinical signs associated with aMPV include snickering, rales, sinusitis, rhinitis, and tracheitis (13). In experimentally infected turkey poults, aMPV has been detected in the cilia of turbinates, trachea, and lung epithelial cells (11,19,20,21). In 1997, the first U.S. aMPV isolate was recovered from commercial turkeys in Colorado following an outbreak of upper respiratory disease (34). The disease emerged in turkey flocks in Minnesota the following year and has continued to spread into neighboring states (28). Currently only one serotype of aMPV has been described; however, nucleotide sequence analysis has identified four subtypes: A, B, and D comprising European isolates, and C from U.S. isolates (4,14,34,35).

Previous efforts to control aMPV in turkey flocks the United States have focused on flock-management practices, controlled exposure, and the development of a commercially licensed live virus vaccine. It has previously been shown that intramuscular DNA vaccination with the fusion (F) and nucleoprotein (N) can reduce clinical signs of disease following challenge, although only the F gene was able to increase virus-neutralization titers (16). Because the F gene contains epitopes necessary to stimulate neutralizing antibodies, this was not unexpected. Interestingly, DNA vaccination with the N gene resulted in decreased VN titers compared to nonvaccinated challenged birds, suggesting the memory B-cell response to challenge preferentially produced antibodies that were not neutralizing (e.g., to the N protein). It has also been shown that mucosal vaccination with an aMPV virosome vaccine resulted in decreased virus titers and clinical signs (15). Virosomes contain functionally active viral proteins, and thus mimic viral infection. Mucosal application with this vaccine was able to induce VN antibody memory response, as challenged birds displayed increased VN titers compared to unvaccinated birds. The use of attenuated live-virus strains of aMPV

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have been tested for efficacy with mixed results (29,30,39). At least one study has reported that although vaccination of turkeys with a cell-culture–propagated subtype C aMPV isolate resulted in milder disease signs upon challenge, the vaccine dose necessary for protection exceeded that required for infection (12).

In European countries, cell-culture attenuated or inactivated vaccines are currently being used to control disease caused by subtypes A and B (13). Cook *et al*. reported that subgroup A and B attenuated vaccines could protect turkeys from clinical signs of disease against the Colorado subtype C isolate; however, it was not reported if vaccination reduced virus load, decreased histopathologic lesions, or increased neutralization antibody titers (6). Although these vaccines have been shown to decrease clinical sings of TRT challenge, one report suggests that attenuated live aMPV vaccines may promote the incidence of disease in young birds (25).

Previous studies with formalin-inactivated pneumovirus vaccines prepared against human respiratory syncytial virus (RSV) and bovine RSV have described an enhanced disease severity following challenge in the presence of antiviral, but nonneutralizing antibodies (8,17,18,24,32). These studies concluded that formalin inactivation disrupted epitopes necessary for induction of virus-neutralizing antibodies and resulted in enhanced disease following challenge. The objectives of this study were to determine if intranasal immunization with an inactivated aMPV subtype C protects turkeys from clinical disease and decreases viral shedding. In addition, the antibody response following vaccination and challenge was determined by ELISA and neutralization assay.

MATERIALS AND METHODS

Virus. The Minnesota (Mn) 1A isolate (kindly provided by Dr. Bruce Seal, Southeast Poultry Research Laboratory, USDA, Athens, GA) of aMPV was used for these studies. The virus was isolated in 1997 from a turkey flock in Minnesota infected with aMPV. The virus was propagated in monolayers of Vero cells grown at 37 C in 5% CO_2 in F12/DMEM with 5% fetal bovine serum and antibiotics (Invitrogen, Baltimore, MD).

Experimental animals. The source of the 1-day-old turkeys was the SEPRL specific-pathogen-free (SPF) Beltsville White turkey flock. Nonchallenged control birds were housed in Horsfall isolation units under negative pressure in biosafety level 2 facilities, and virus-challenged birds were housed in similar units in a biosafety level 3 agriculture facility (3). Birds received feed and water *ad libitum*.

Vaccine preparation. aMPV was grown in Vero cells as described above in T-75 tissue-culture flasks (Costar, Corning, NY). After 5–7 days of growth, flasks were frozen at −70 C, thawed, and refrozen two additional times. Cells were scraped and the resulting cell mixture centrifuged (7000 × *g*) for 30 min at 4 C. The pellet containing cellular debris was discarded. Virus from the supernatant was recovered by centrifugation (24,000 × *g*) for 2 hr. The virus was resuspended in 1 ml of F12/DMEM and placed over a discontinuous sucrose gradient (0%–45%). Centrifugation was carried out for 18 hr (22,000 × *g*), and the resulting virus band was extracted via needle and syringe. Virus was washed as above and resuspended in 1 ml of phosphate-buffered saline (PBS; pH 7.4).

Virus inactivation with formalin (Sigma Chemical Co., St. Louis, MO) was carried out as previously described (2). To ensure complete inactivation, a sample of inactivated virus was safety tested by three serial passages in Vero cells. Protein concentration was determined with the use of bicinchoninic acid (BCA) method (Pierce, Rockford, IL) (36).

To examine the effect of adjuvant on mucosal immunity, one group of birds received formalin-inactivated aMPV formulated with L121 (BASF, Mt. Olive, NJ) (1.25% v/v). This adjuvant has previously been shown to enhance antibody formation (1,10,37).

Experimental design. Fifty-four 1-day-old SPF turkey poults were randomly divided into six groups of nine birds. All vaccinations were given intranasally (IN) in 0.1-ml aliquots, one-half volume per nare. Birds in groups 1 and 4 received PBS at days 1 and 14 of age. Birds in group 2 and 5 received 50 μg of inactivated aMPV on day 1 and 100 μg on day 14. Birds in groups 3 and 6 received 50 and 100 μg of inactivated aMPV formulated with L121 adjuvant on days 1 and 14, respectively. Birds from groups 1, 2, and 3 served as unchallenged controls, and birds in groups 4, 5, and 6 were challenged via the IN route with sucrose-purified Mn 1A, 1×10^5 $TCID_{50}$ per bird at 21 days old.

Serum was taken from each bird on days 0, 7, and 14 postchallenge (pc) and tested for antibodies to aMPV by ELISA and virus-neutralization assays. Three birds from each group were euthanatized at 2, 7, and 14 days postchallenge. At necropsy clinical signs were recorded for each bird and included nasal exudates, snickering, swollen head, and swollen sinuses. Samples (tracheal swabs and lung tissue) were collected for virus detection by aMPV-specific reverse transcription (RT) polymerase chain reaction (PCR), virus isolation and histopathology. In Expt. II, the experiment was repeated as in Expt I.

aMPV ELISA. Serum IgG and IgA anti-aMPV antibodies were determined by ELISA as previously described, with minor alterations (15). Either goat antiturkey IgG or goat antichicken IgA (both 1:1000 dilution in blocking buffer) conjugated to horseradish peroxidase (Southern Biotechnology Associates, Inc., Birmingham, AL) was added to each well for detection of IgG or IgA antibodies, respectively. Previous studies have determined cross reactivity of antichicken IgA antibodies with turkey IgA (38). Recorded optical densities (OD) at 490-nm wavelength were determined by subtracting mock-coated wells from aMPV-coated wells.

Virus neutralization. Serum antibodies to aMPV were tested for virus neutralization (VN) with the use of Vero cells grown in 96-well, flat-bottomed microtiter plates (Costar) as previously described (16). Briefly, serum samples were diluted twofold and tested in triplicate. Positive and negative serum controls were added to each group of plates tested. Vero cells were monitored for cytopathic effect (CPE) for 7 days postinoculation. The VN titer was expressed as $\log_2$ of the reciprocal of the highest dilution that completely inhibited cytopathic effect.

Virus isolation. Virus isolation (VI) from tracheal swabs taken from each bird was performed as previously described (16). Briefly, swabs were placed in 1 ml PBS, vortexed, and filter sterilized (0.22 μm); and 100 μl were placed on Vero cell monolayers. The Vero cells were allowed to grow for 5 days as described above. The plates were frozen at −80 C and thawed three times and the supernatants transferred to fresh Vero cells. Typical aMPV syncytia formation on Vero cells indicated a positive aMPV isolation, which was confirmed by RT-PCR. All samples were passed individually up to five times.

Virus detection with RT-PCR. For RT-PCR, RNA was extracted from homogenized tissues with a RNA extraction kit (Qiagen, Valencia, CA) according to manufacturer's directions. Primers were designed to the F gene with known sequences (GenBank Accession No. AF187153) with OLIGO™ version 4.0 software (National Bioscience, Plymouth, MN) as previously described (15).

Histopathology. Tissue samples of lung and distal trachea (distal end) from each bird were fixed by immersion in 10% neutral buffered formalin, routinely processed, and paraffin embedded. Serial sections of 2–5 μm thick were stained with hematoxylin and eosin. According to the method of Perkins and Swayne, histologic lesions were scored by severity as follows: (−) = no lesions, (+) = minimal, (++) = moderate, (+++) = severe (31). Slides of tissues were scored by an avian pathologist who did not have prior knowledge of groupings.

Statistical analysis. Statistical analysis of the data was performed with the use of ANOVA with either the Tukey-Kramer HSD for ELISA comparisons or the Student-Newman-Keuls for VN titer comparisons utilizing JMP® statistics software (SAS Institute, Inc., Cary, NC). P values ≤ 0.05 were considered to be significant.

RESULTS

Clinical signs following aMPV challenge. Following intranasal vaccinations, birds were divided into aMPV-challenged and

Table 1. Clinical signs and virus detection by RRT-PCR from lung and virus isolation from tracheal swabs from birds immunized with formalin-inactivated Minnesota 1a (Expts. I and II).

Group	d.p.c.[A]	Morbidity[B] (no. sick/total)	Virus detection[C] by Lung[D]	Virus detection[C] by Tracheal swabs[E]
1—Control	2	0/6	0/6	0/6
nonchallenged	7	0/6	0/6	0/6
	14	0/6	0/6	0/6
2—PBL-Mn	2	0/6	0/6	0/6
nonchallenged	7	0/6	0/6	0/6
	14	0/6	0/6	0/6
3—PBL-Mn-L121	2	0/6	0/6	0/6
nonchallenged	7	0/6	0/6	0/6
	14	0/6	0/6	0/6
4—Control	2	6/6	0/6	5/6
challenged	7	4/6	0/6	6/6
	14	0/6	0/6	2/6
5—PBL-Mn	2	5/6	6/6	6/6
challenged	7	3/6	0/6	6/6
	14	0/6	2/6	2/6
6—PBL-Mn-L121	2	4/6	5/6	5/6
challenged	7	2/6	0/6	6/6
	14	0/6	0/6	2/6

[A]d.p.c. = days pc.
[B]Number of birds displaying clinical signs of aMPV.
[C]Number of aMPV-positive samples/number of samples tested.
[D]RNA extracted from lung tissue at each day indicated and used in RT-PCR with aMPV F gene specific primers. + = positive for aMPV, − = negative for aMPV.
[E]Following passage on Vero cell monolayers and confirmed by RT-PCR specific for aMPV.

-nonchallenged groups. Results from Expts. I and II were similar and the data were pooled. As expected, nonchallenged groups (1–3) did not exhibit clinical signs of aMPV disease (Table 1). Mild clinical signs were seen in all virus-challenged groups on day 2 postchallenge, which normally consisted of a mild snick and/or nasal discharge (Table 1). Although minor differences in morbidity were observed between the groups, the incidence of morbidity was not significantly different between vaccinated (groups 5 and 6) and nonvaccinated (group 4) birds.

Serum immune response to aMPV. A purified-virus ELISA was used to detect humoral IgG and IgA responses to aMPV. As seen in Fig. 1, all birds from nonchallenged groups (1–3) did not produce significant increases in humoral IgG or IgA to aMPV, regardless of vaccination status. In aMPV-challenged groups, no detectable serum IgG or IgA was observed by ELISA at day 0 postchallenge. However, by day 7 postchallenge all groups (4–6) displayed increases in IgG to aMPV, and ELISA levels were significantly different ($P \leq 0.05$) from nonvaccinated nonchallenged in birds from group 1. At day 14, all virus-challenged groups showed marked increases in IgG and IgA to aMPV, which were significantly different ($P \leq 0.05$) than nonchallenged birds. No differences in serum antibody response levels were observed between nonvaccinated (group 4) and vaccinated (groups 5 and 6) birds following challenge.

Virus neutralization. Unchallenged groups of turkeys did not have detectable VN titers on all days tested (groups 1–3) (Table 2). Serum VN titers ($\log_2$) were determined to be highest in unvaccinated virus-challenged birds (group 4), which increased from <2 on day 0 to 5.0 on day 7, and 6.0 by day 14. Birds vaccinated with formalin-inactivated aMPV (groups 5 and 6) also increased VN titers during the course of the experiment; however, the levels were decreased compared to the unvaccinated aMPV-challenged birds (group 4), and did not go above 3.5 on either day 7 or 14. Significant differences in serum VN titers were observed between unvaccinated (group 4) and vaccinated (groups 5 and 6) birds when challenged with aMPV on both day 7 and 14, with unvaccinated birds displaying increased VN titers compared to vaccinated birds.

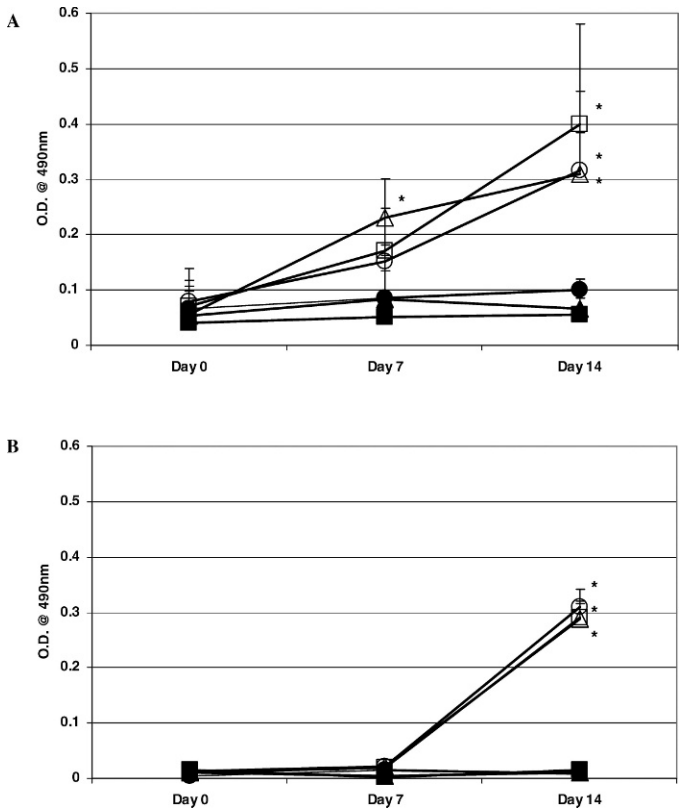

Fig. 1. Serum IgG (A) and IgA (B) response of turkeys to aMPV following intranasal immunization from nonchallenged and aMPV-challenged groups. Birds were immunized at 1 and 14 days of age with formalin-inactivated aMPV and challenged at 21 days of age with 1×10^5 $TCID_{50}$ aMPV. Serum samples were taken at 0, 7, and 14 days postchallenge, diluted 1:500 and tested by ELISA for antibodies to aMPV. The results are expressed as optical density of aMPV-coated wells minus mock-coated wells. Each sample indicates the mean from each group of birds ($n = 6$) on each day tested. Symbols for groups: ■—group 1, control nonchallenged; ▲—group 2, immunized with formalin-aMPV, nonchallenged; ●—group 3, immunized with formalin-aMPV containing L121, nonchallenged; □—group 4, control aMPV challenged; △—group 5, immunized with formalin aMPV, aMPV challenged; ○—group 6, immunized with formalin-aMPV containing L121, aMPV-challenged. * = Significantly different from nonvaccinated nonchallenged group.

Virus isolation from tracheal swabs. Tracheal swabs were taken from each bird and passed up to five times in Vero cells for aMPV isolation. No virus was isolated from nonchallenged birds from tracheal swabs (Table 1). aMPV was isolated on Vero cells from all but two tracheal swabs at day 2 pc, and all swabs at day 7 pc. At day 14, 33% of swabs were positive by VI from all groups. Detection of aMPV RNA in lung tissue was only observed in birds receiving inactivated aMPV (groups 5 and 6). No viral RNA was detected in unvaccinated challenged birds (group 4) on any day sampled (Table 1).

Histopathology. Table 3 describes the severity of histopathologic lesions in the trachea, lung, and bronchi following virus challenge in Expts. I and II. No lesions were seen in unchallenged groups (groups 1–3) on all days tested (Fig. 2a). Vaccination with inactivated aMPV

Table 2. Virus neutralization titers[A] to aMPV following intranasal vaccination and challenge with Minnesota 1a (Expts. I and II).

Group	Day 0	Day 7[B]	Day 14
Control			
1—Nonchallenged	<2	<2	<2
4—Challenged	<2	5.0[a]	6.0[a]
PBL-Mn			
2—Nonchallenged	<2	<2	<2
5—Challenged	<2	3.0[b]	3.5[b]
PBL-Mn-L121			
3—Nonchallenged	<2	<2	<2
6—Challenged	<2	2.0[b]	3.5[b]

[A]Reciprocal of the highest dilution (log_2) that completely inhibited the viral cytopathogenic effect on Vero cells. Results indicate mean from each group from two independent experiments. Initial serum dilution 1:4.

[B]Titers within columns with different letters are significantly different ($P < 0.05$). Titers less than 2 were not tested.

did not lessen histopathologic lesions compared to nonvaccinated birds following virus challenge. All lesions observed in the trachea and lungs of groups receiving virus challenge were mild. The most common lesion in the trachea in unvaccinated virus-challenged birds (group 4) was mild deciliation of epithelial cells with mild heterophilic inflammation on days 2 and 7. No other lesions were observed in the trachea on day 14 in this group. In contrast, vaccinated virus-challenged birds (groups 5 and 6) displayed individual cell necrosis and loss of cilia, with mild focal epithelial attenuation on days 7 and 14 postchallenge. The lungs of nonvaccinated birds (group 4) displayed mild congestion and minimal heterophilic inflammation, whereas vaccinated birds (groups 5 and 6) exhibited minimal to mild interstitial heterophilic to mononuclear inflammation. In the bronchi, only mild lymphoid hyperplasia was observed in unvaccinated virus-challenged birds on each day tested (group 4) (Fig. 2b). Birds in groups 5 and 6 displayed minimal to mild heterophilic bronchitis and mild to moderate reactive lymphoid hyperplasia with hemorrhage following virus challenge (Fig. 2c,d). The addition of adjuvant to the vaccine had no effect on lesions observed in aMPV-vaccinated virus-challenged groups.

DISCUSSION

In an effort to prevent aMPV disease and virus replication, mucosal vaccination with inactivated aMPV was evaluated. The vaccine did not appear to decrease incidence or duration of clinical signs, histologic lesions, or virus isolation following challenge compared to unvaccinated birds. The inclusion of adjuvant also did not appear to enhance protection or immunity. Interestingly, vaccination with inactivated aMPV resulted in decreased VN antibody responses following virus challenge compared to unvaccinated challenged birds.

These results display some similarities to previous studies testing inactivated *Pneumoviridae* vaccines, in that decreased neutralizing antibody responses are observed when animals receiving formalin-inactivated vaccine virus are exposed to challenge virus (22,27,33). It has since been established that formalin inactivation of the hRSV disrupts epitopes on the F and G surface proteins necessary to elicit neutralizing antibodies (23). It is possible that inactivation of aMPV may disrupt epitopes necessary for induction of neutralizing antibodies, although we did not examine the mechanism for this observation in these studies. Interestingly, Naylor *et al.* (26) demonstrated that maternal antibodies from dams receiving inactivated TRT vaccine did not protect poults against subsequent virus challenge, although VN responses were not tested. Mucosal vaccination with inactivated aMPV also resulted in a slight increase of bronchial lesions based on histopathology. The turkeys vaccinated with only inactivated aMPV (group 5) displayed a mild increase in pulmonary lesions, observed as mild bronchitis and reactive lymphoid hyperplasia with hemorrhage, in the bronchi in both Expts. I and II. Previous vaccine studies with inactivated hRSV and bRSV indicated an enhanced disease pathology following vaccination and infection as compared to nonvaccinated individuals (8,18). Future research with greater numbers of animals should provide further insight into this phenomenon in turkeys.

Two common characteristics of aMPV infection of turkeys are the lack of clinical signs in uncomplicated cases of disease and the severe increase in morbidity and mortality associated with the presence of secondary pathogens (11). In the present study, the clinical signs in the virus-challenged groups were mild in nature. Although snickering was observed in these birds, we did not observe rales, sinusitis, or rhinitis, signs normally associated with field cases. These results may not be unexpected, because uncomplicated infection with aMPV has been shown to result in mild disease and the tests were conducted in SPF turkeys.

In terms of flock management, our studies show that despite increased antibody levels, protection against virus challenge was not conferred. Because flock serology is usually tested by nonfunctional analysis (e.g., ELISA), immune status following vaccination may not be protective. Future field studies should examine the relationship between ELISA and VN when inactivated vaccines are used.

Table 3. Average severity of histologic[A] lesion scores in trachea, lung, and bronchi following challenge with aMPV/Minnesota 1a in Expts. I and II.

		Trachea		Lung		Bronchi	
Group	d.p.c.[B]	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II
4—Control	2	+	+	+	+	+	+
	7	+	+	−	+	+	+
	14	−	−	+	+	+	+
5—BPL-Mn	2	−	−	+	+	+	+
	7	+	+	+	+	++	++
	14	+	+	+	+	++	++
6—BPL-Mn-L121	2	−	−	−	−	++	++
	7	+	+	+	+	+	++
	14	+	+	−	−	−	−

[A]− = no lesions; + = mild; ++ = moderate; +++ = severe.

[B]d.p.c. = days pc.

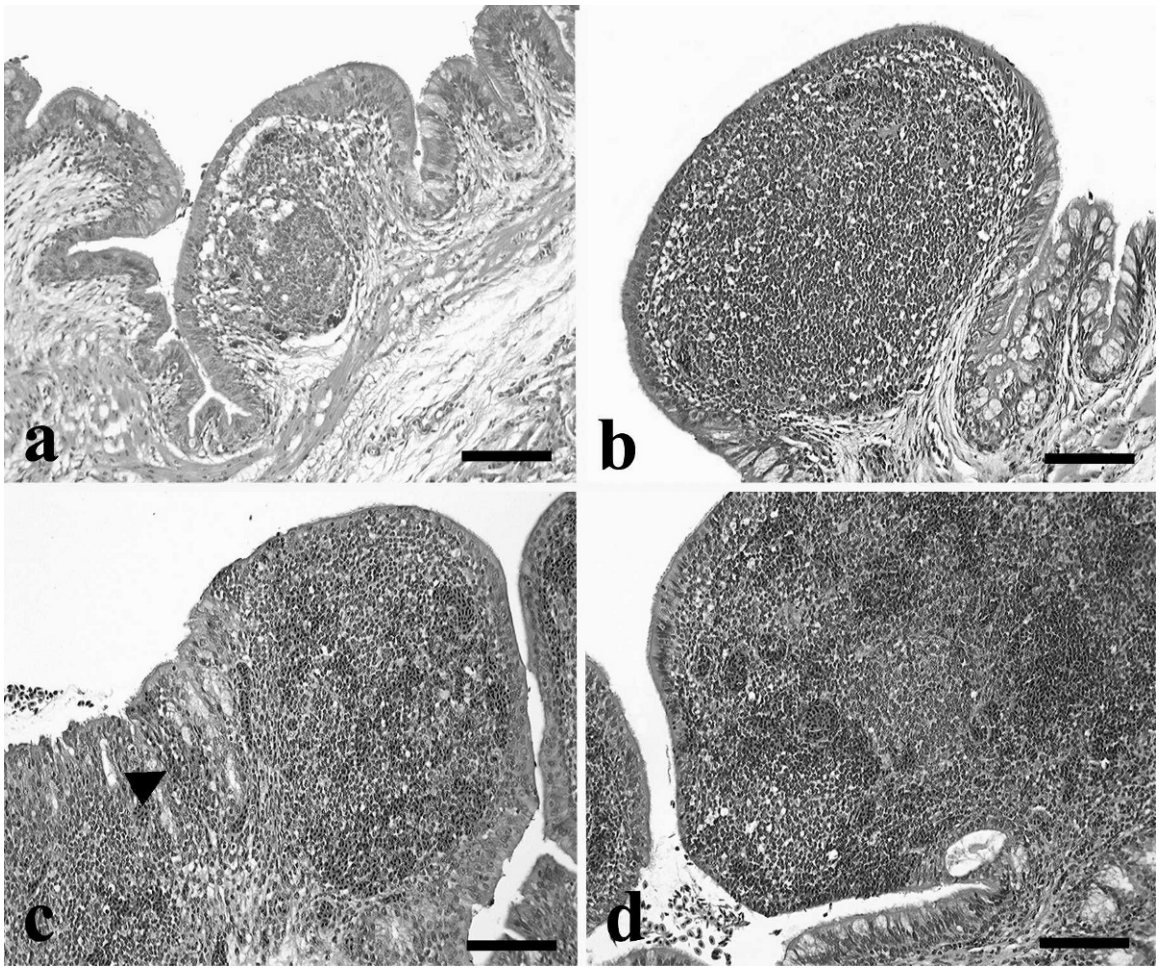

Fig. 2. Bronchi histopathology of SPF turkeys 7 days following aMPV challenge in Expt. I. All sections were formalin fixed, paraffin embedded, and stained with hematoxylin and eosin. (a) Tissue from normal lung (group 1). (b) Unimmunized turkey challenged intranasally at 3 wk of age with 1×10^5 $TCID_{50}$ aMPV showing mild lymphoid hyperplasia (group 4). (c) Turkey immunized intranasally with formalin-inactivated aMPV at 1 and 14 days of age, then challenged intranasally at 3 wk of age with aMPV (group 5). Bronchiolar lesions include mild to moderate focal epithelial hyperplasia with heterophilic infiltrates (arrow) and hemorrhage. (d) Turkey immunized intranasally with formalin-inactivated aMPV formulated with L121 at 1 and 14 days of age, then challenged intranasally with aMPV at 3 wk of age. Bronchiolar lesions include mild to minimal heterophilic bronchitis and mild to moderate reactive lymphoid hyperplasia with hemorrhage (group 6). Bar = 100 μm.

In conclusion, these studies provide important information on mucosal application of inactivated aMPV subtype C, in that neither a decrease from viral infection or histopathologic lesion severity was observed following virus infection. Although it remains unknown whether inactivated vaccines made from different aMPV subtypes produce neutralizing antibodies following vaccination and challenge, current field strategies for aMPV vaccine application include the use of both inactivated and live vaccines against the A and B subtypes (7). It also remains unclear what impact the age of vaccination has on inducing mucosal immunity in turkeys. It is possible that the birds were immunologically immature in terms of mucosal processing during vaccination. Finally, it is recommended that inactivated vaccines for aMPV should be tested for their ability to induce virus-neutralizing antibodies as well as to protect from disease and decrease virus replication.

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